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Dated 07 JUL 2000

Patents Form 1/77

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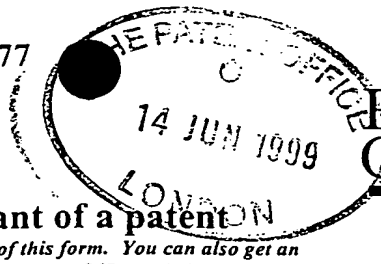

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14 JUN 1999

15JUN99 E454587-3 D00027
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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

9913823.2

The Patent Office
Cardiff Road
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1. **Your reference** — 44.3.70304/001

2. **Patent application number** *(The Patent Office will fill in this part)*

3. **Full name, address and postcode of the or of each applicant** *(underline all surnames)*

Proteus Molecular Design Limited
Beechfield House
Lyme Green Business Park
Macclesfield
Cheshire
SK11 0JL

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give country/state of incorporation — UK 05053142003

4. **Title of the invention** — Compounds

Form 5.177 5161

5. **Name of your agent** *(if you have one)* — Frank B. Dehn & Co.

Marken & May 2 ...

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

179 Queen Victoria Street
London
EC4V 4EL

13 Queen Victoria Str...
Macclesfield
Cheshire
SK11 6LP

Patents ADP number *(if you know it)* — 166001

6. **If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and** *(if you know it)* **the or each application number**

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
	9710553.1	

7. **If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application**

Number of earlier application	Date of filing *(day / month / year)*

8. **Is a statement of inventorship and of right to grant of a patent required in support of this request?** *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

yes

70304/000.604

Compounds

This invention relates to compounds which are inhibitors of serine proteases and to pharmaceutical compositions thereof and their use in the treatment of the human or animal body.

The serine proteases are a group of proteolytic enzymes which have a common catalytic mechanism characterized by a particularly reactive Ser residue. Examples of serine proteases include trypsin, tryptase, chymotrypsin, elastase, thrombin, plasmin, kallikrein, Complement C1, acrosomal protease, lysosomal protease, cocoonase, α-lytic protease, protease A, protease B, serine carboxypeptidase II, subtilisin, urokinase, Factor VIIa, Factor IXa, and Factor Xa. The serine proteases have been investigated extensively over a period of several decades and the therapeutic value of inhibitors of serine proteases is well understood.

Serine protease inhibitors play a central role in the regulation of a wide variety of physiological process including coagulation, fibrinolysis, fertilization, development, malignancy, neuromuscular patterning and inflammation. It is well known that these compounds inhibit a variety of circulating proteases as well as proteases that are activated or released in tissue. It is also becoming clear that serine protease inhibitors inhibit critical cellular processes, such as adhesion, migration, free radical production and apoptosis. In addition, animal experiments indicate that intravenously administered serine protease inhibitors, variants or cells expressing serine protease inhibitors, provide a protective effect against tissue damage.

Serine protease inhibitors have also been predicted to have potential beneficial uses in the treatment of disease in a wide variety of clinical areas such as

oncology, neurology, haematology, pulmonary medicine, immunology, inflammation and infectious disease.

In particular serine protease inhibitors may be beneficial in the treatment of thrombotic diseases, asthma, emphysema, cirrhosis, arthritis, carcinoma, melanoma, restenois, atheroma, trauma, shock and reperfusion injury.

Thus for example an inhibitor of Factor Xa has value as a therapeutic agent as an anticoagulant, e.g. in the treatment and prevention of thrombotic disorders. The use of a Factor Xa inhibitor as an anticoagulant is desirable in view of the selectivity of its effect. Many clinically approved anticoagulants have been associated with adverse events owing to the non-specific nature of their effects on the coagulation cascade.

Also, there are well-known associations of α1 protease inhibitor deficiency with emphysema and cirrhosis and C1 esterase inhibitor deficiency with angioedema.

We have now found that certain aromatic compounds carrying bulky lipophilic side chains are particularly effective as inhibitors of serine proteases, especially proteases with negatively charged P1 specificity pockets, and most especially the serine proteases thrombin, trypsin, urokinase, Factor VIIa and most importantly Factor Xa. The Factor Xa inhibitors of this invention are potentially useful for the prophylaxis or treatment of thrombotic disorders such as amongst others venous thrombosis, pulmonary embolism, arterial thrombosis, myocardial ischaemia, myocardial infarction, and cerebral thrombosis. They potentially have benefit in the treatment of acute vessel closure associated with thrombolytic therapy and restenosis, e.g. after transluminal coronary angioplasty or bypass grafting of the coronary or peripheral arteries and in the maintenance of vascular access patency in long term hemodialysis patients.

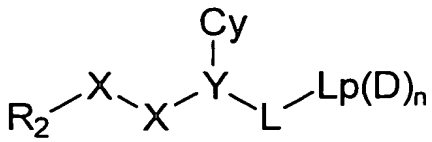
Factor Xa inhibitors of this invention may, with benefit, form part of a combination therapy with an anticoagulant with a different mode of action or with a thrombolytic agent.

We have previously reported in WO99/11657 and WO99/11658 that certain benzamidine and isoquinoline derivatives carrying a bulky lipophilic side chain are excellent inhibitors of serine proteases. Surprisingly, we have now found certain other aromatic compounds also show inhibitory activity against serine proteases, in particular Factor Xa, despite the lack of the amidino or 1-aminoisoquinoline functionality previously believed to be crucial for activity as a factor Xa inhibitor.

The compounds of the invention are thus likely to be available for administration orally. Also, it has been found that the compounds of the invention perform excellently in the prothrombin time assay (PT) when compared to aminoisoquinolines of similar factor Xa activity. The PT assay is a coagulation assay and it is widely accepted that direct acting Factor Xa inhibitors which perform well in the PT assay are more likely to be good antithrombotics.

In WO99/09053 certain 2-aminobenzamide compounds are disclosed as potential motilin receptor antagonists and in US 3268513 similar 2-aminobenzamide compounds are suggested as potential antibacterial agents. However, the novel compounds of the present invention have not before been suggested as potential serine protease inhibitors.

Thus viewed from an one aspect the invention provides a serine protease inhibitor compound of formula (I)



(I)

(where R_2 represents a 5 or 6 membered aromatic carbon ring optionally interrupted by a nitrogen, oxygen or sulphur ring atom, optionally being substituted in the 3 or 4 position by halo, nitro, haloalkoxy, amino, cyano, haloalkyl, alkylthio, alkenyl, alkynyl, acylamino or R_1 or the substituents at the 3 and 4 positions taken together form a fused ring which is a 5 or 6 membered carbocyclic or heterocyclic ring optionally substituted by halo, haloalkoxy, haloalkyl, cyano, nitro, amino, hydrazido, alkylthio, alkenyl, alkynyl or R_1, and optionally substituted in the position alpha to the X-X.. group (i.e. 6 position for a six membered aromatic ring etc) by amino, hydroxy, halo, alkyl, alkoxy or alkylthio with the proviso that R_2 cannot be isoquinolyl;

each X independently is a C, N, O or S atom or a CO, CR_1, $C(R_1)_2$ or NR_1 group, at least one X being C, CO, CR_1 or $C(R_1)_2$;

each R_1 independently represents hydrogen or hydroxyl, alkoxy, alkyl, aminoalkyl, hydroxyalkyl alkoxyalkyl, alkoxycarbonyl, acyloxymethoxycarbonyl or alkylamino optionally substituted by hydroxy, alkylamino, alkoxy, oxo, aryl or cycloalkyl;

L is an organic linker group containing 1 to 5 backbone atoms selected from C, N, O and S, or a branched alkyl or cyclic group;

Y (the α-atom) is a nitrogen atom or a CR_1 group or Y and L taken together form a cyclic group;

Cy is a saturated or unsaturated, mono or poly cyclic, homo or heterocyclic group, preferably containing 5 to 10 ring atoms and optionally substituted by groups R_3 or phenyl optionally substituted by R_3;

each R_3 independently is R_1, amino, halo, cyano, nitro, thiol, alkylthio, alkylsulphonyl, alkylsulphenyl, triazolyl, imidazolyl, tetrazolyl, hydrazido, alkyl imidazolyl, thiazolyl, alkyl thiazolyl, alkyl oxazolyl, oxazolyl, alkylsulphonamido, alkylamino-sulphonyl, aminosulphonyl, haloalkoxy and haloalkyl;

Lp is a lipophilic organic group, e.g. an alkyl, heterocyclic, alkenyl, alkaryl, cycloalkyl, polycycloalkyl, cycloalkenyl, aryl, aralkyl or haloalkyl group or a combination of two or more such groups optionally substituted by one or more of oxa, oxo, aza, thia, or R_3 groups, preferably a group containing up to 25 carbon atoms;

D is a hydrogen bond donor group; and n is 0, 1 or 2);

or a physiologically tolerable salt thereof, e.g. a halide, phosphate or sulphate salt or a salt with ammonium or an organic amine such as ethylamine or meglumine.

In the compounds of the invention, where the alpha atom is carbon it preferably has the conformation that would result from construction from a D-α-aminoacid NH_2-$CR_1(Cy)$-COOH where the NH_2 represents part of X-X. Likewise the fourth substituent R_1 at an alpha carbon is preferably a methyl or hydroxymethyl group or hydrogen.

In the compounds of the invention, unless otherwise indicated, aryl groups preferably contain 5 to 10 ring atoms optionally including 1, 2 or 3 heteroatoms selected from O, N and S; alkyl, alkenyl or alkynyl groups or alkylene moieties preferably contain up to 6 carbons, e.g. C_{1-6} or C_{1-3}; cyclic groups preferably have ring sizes of 3 to 8 atoms; and fused multicyclic groups preferably contain 8 to 16 ring atoms.

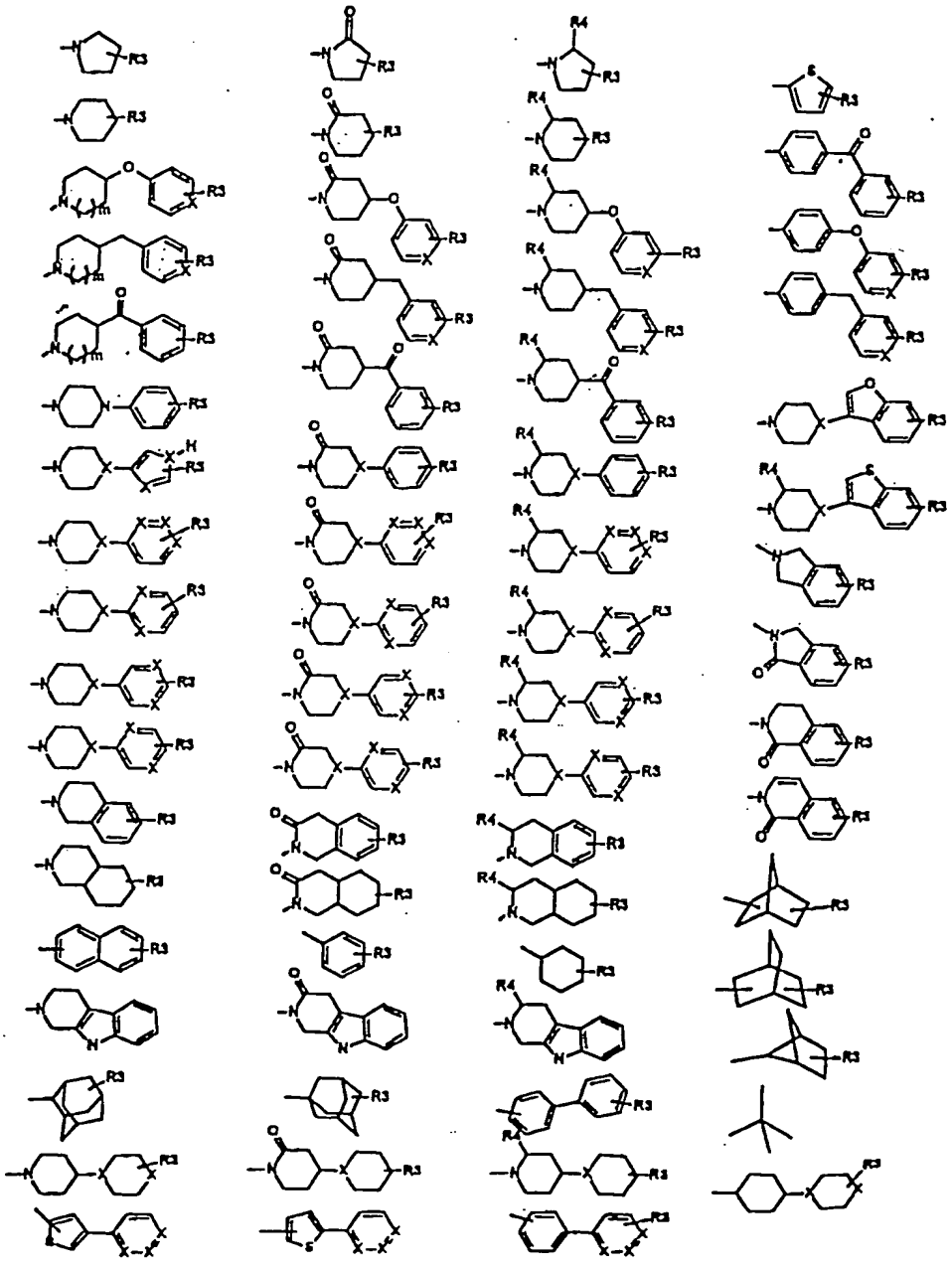
The linker group from the R_2 group to the alpha atom is preferably selected from -CH=CH-, -CONH-, -$CONR_1$-, -NH-CO-, -NH-CH_2-, -CH_2-NH-, -CH_2O-, -OCH_2-, -COO-, -OC=O- and -CH_2CH_2-. Preferably, the X moiety nearest to the alpha atom is an NH or O atom, most preferably a NH group. The X moiety alpha to the aromatic ring is preferably a carbon based group such as CH_2 or CO, preferably CO. Thus a particularly preferred linker X-X is -CONH-. In an alternative embodiment the linker is preferably a -OCH_2- group.

The alpha atom (Y) is preferably a CH or $C(CH_3)$ group, especially CH.

The linker group from the alpha atom to the lipophilic group is preferably CO, CH_2NH, $CONR_1(CH_2)_m$, $(CH_2)_mN(R_1)CO(CH_2)_m$, $(CH_2)_{m+2}$, $CO(CH_2)_m$, $(CH_2)_mCO$, $(CH_2)_mOC=O$, $(CH_2)_mO$, $CH=CH(CH_2)_m$, SO_2, SO_2NR_1, $SO_2(CH_2)_m$, $(CH_2)_mSO_2$ or $(CH_2)_mSO_2NR_1$ (where each m is independently 0 or 1). The linker may be optionally branched, for example, to incorporate a polar functionality. In a preferred embodiment Y and L taken together form a cyclic group and the alpha atom is therefore a carbon atom. The cyclic group can be unsubstituted or substituted and can have a ring size of from 3 to 8 atoms. Preferably, the cyclic group is a cyclic amide, most preferably wherein the amide nitrogen of the cyclic amide group is bound to the lipophilic group.

The lipophilic group preferably comprises a cycloalkyl, azacycloalkyl, diazacycloalkyl, phenyl, naphthyl, adamantyl, decalinyl, tetrahydrodecalinyl, bicycloalkyl, mono- or diazabicycloalkyl, mono- or bicyclo heteroaromatic or a linear or branched alkyl, alkylene, alkenyl or alkenylene group all optionally substituted by one or more groups R_3, or a combination of at least two such groups linked by a spiro linkage or a single or double bond or by C=O, O, S, SO, SO_2, $CONR_1$, NR_1-CO-, NR_1 linkage. For example, representative lipophilic groups include a methyl-cyclohexyl, methylcyclohexylmethyl, methylphenylmethyl, phenylethyl, benzylpiperidinyl, benzoylpiperidinyl, bispiperidinyl or phenylpiperazinyl.

Most preferably, the lipophilic group is selected from



wherein R_3 is as hereinbefore defined;

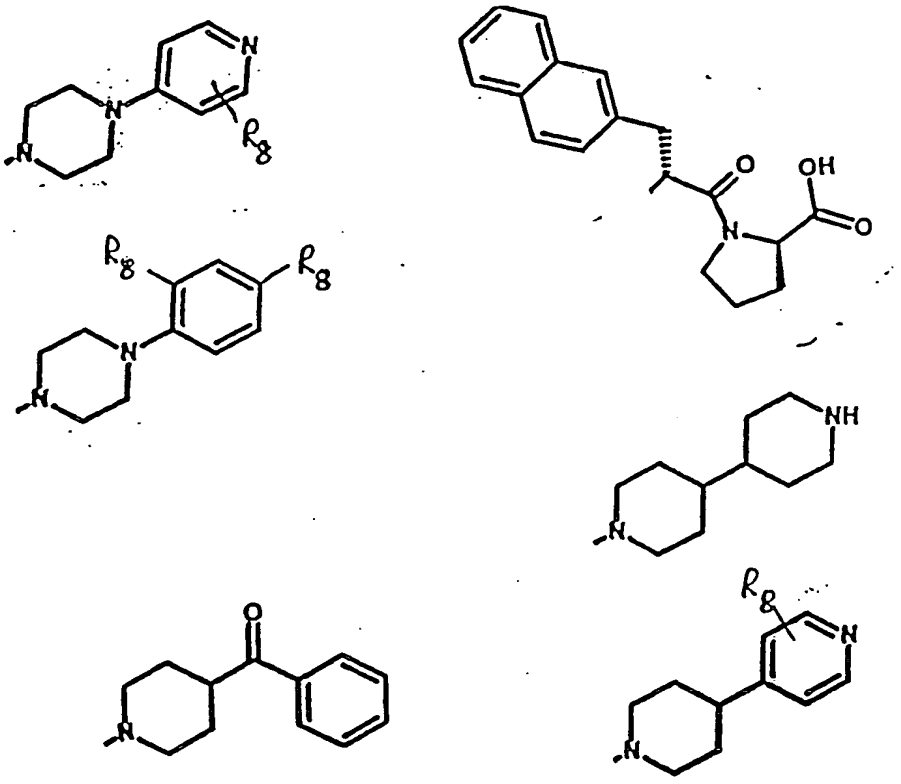
m represents 0 or 1;

R_4 represents hydrogen, $(CH_2)_wCOOH$, $(CH_2)_wCONH_2$, $(CH_2)_wCONα$-AminoAcid;

w represemts an integer from 0 to 4; and

X represents CH or N.

For example specific lipophilic groups include



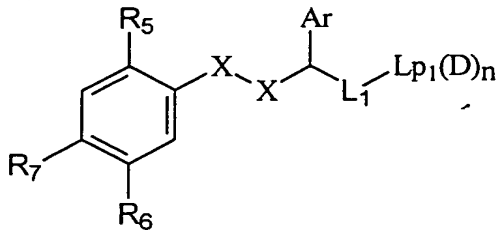
especially when R_8 represents H, OMe, SO_2Me, F, NO_2, $SO_2N(R_1)_2$, Cl, OH or a 5 membered heterocyclic group.

The hydrogen bond donor group which may be attached to the lipophilic group preferably has a nitrogen or oxygen atom as the donor atom and conveniently is a hydroxyl group, a primary, secondary or tertiary amine, or a primary or secondary imine group (as part of an amidine or guanidine) or a saturated or unsaturated heterocyclic group containing a ring nitrogen, preferably a group containing 5 to 7 ring atoms. Where

the donor atom is a ring nitrogen, the remote portion of the heterocyclic ring may be part of the lipophilic group.

The cyclic group attached to the alpha carbon is preferably an optionally R_3 substituted phenyl, thienyl or naphthyl group.

In one embodiment the aromatic R_2 group is an optionally substituted phenyl, naphthyl, indolyl or isoindolyl group and accordingly, preferred compounds of the invention are of formula (II)



(II)

(wherein R_5 is amino, hydroxy or hydrogen, and R_6 and R_7 which may be the same or different represent , halo, nitro, thiol, cyano, haloalkyl, haloalkoxy, amido, hydrazido, amino, alkylthio, alkenyl, alkynyl or R_1 or taken together form a 5 or 6 membered fused carbocyclic ring or 5 membered heterocyclic ring, which may itself be substituted by R_1, amino, halo, cyano, nitro, thiol, alkylthio, haloalkyl, haloalkoxy.

Ar is an unsubstituted or substituted aryl group, preferably phenyl;

X-X is -CONH-, $-CH_2CH_2-$, CH_2O-, -COO-, $-CH_2NH-$, $-OCH_2-$ or $-NHCH_2-$, especially -CONH-;

L_1 is a valence bond or an organic linker group containing 1 to 4 backbone atoms selected from C, N, O and S;

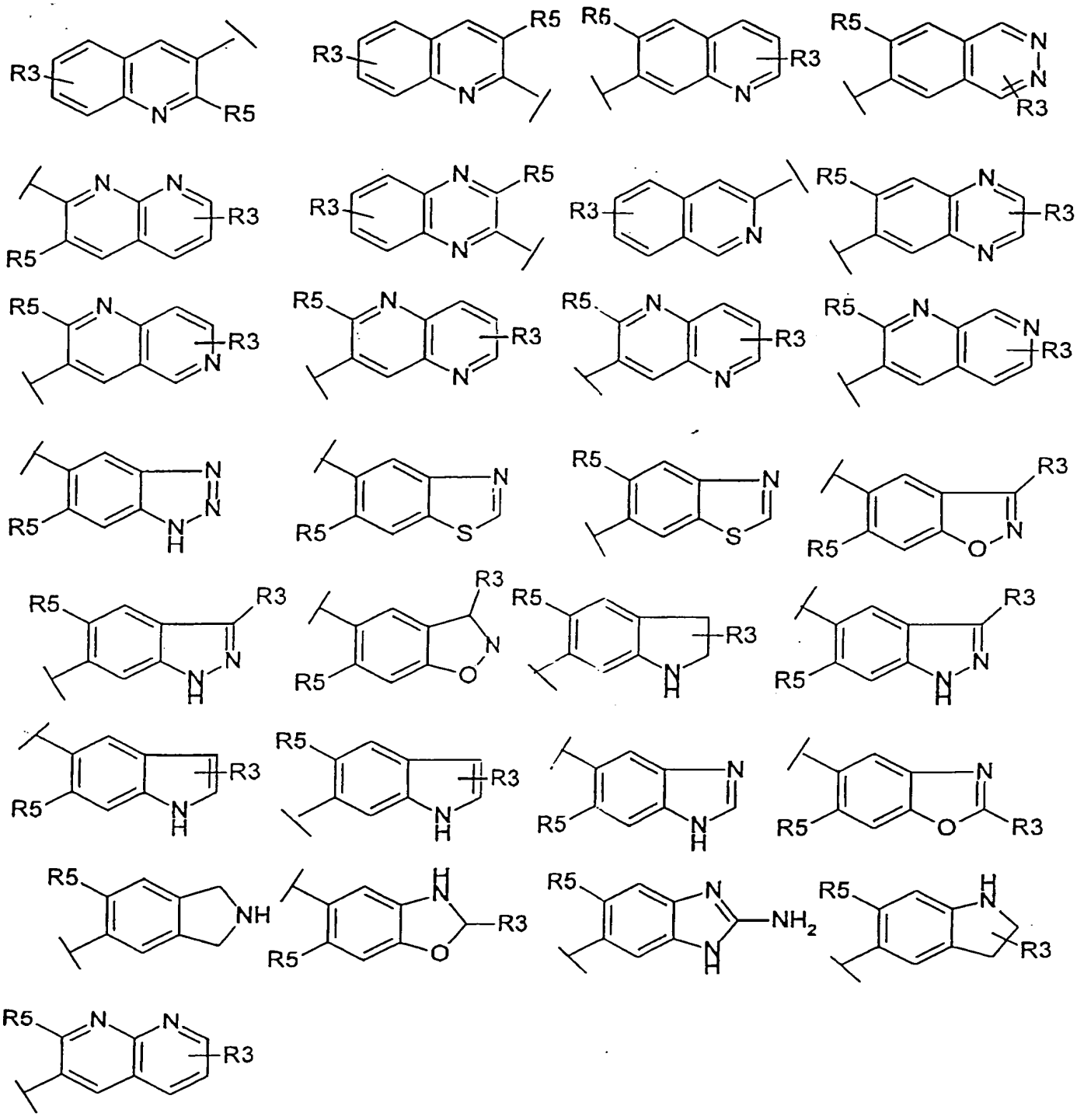
Lp_1 is a cycloalkyl, azacycloalkyl, diazacycloalkyl, phenyl, naphthyl, adamantyl, decalinyl, tetrahydrodecalinyl, bicycloalkyl, mono- or diazabicycloalkyl, mono- or bicyclo heteroaromatic or a linear or branched alkyl, alkylene, alkenyl or

alkenylene group all optionally substituted by a group R_3, or a combination of at least two such groups linked by a spiro linkage or a single or double bond or by C=O, O, S, SO, SO_2, $CONR_1$, NR_1-CO-, NR_1 linkage. For example, representative lipophilic groups include a methylcyclohexyl, methylcyclohexylmethyl, bispiperidinyl, methylphenylmethyl, phenylethyl, benzylpiperidinyl, benzoylpiperidinyl or phenylpiperazinyl and those as hereinbefore described;

D is a hydrogen bond donor group;

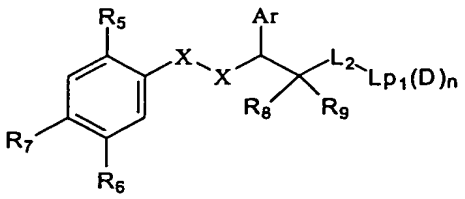
and n is 0, 1 or 2).

In an alternative embodiment the phenyl derivative forming part of the R_2 functionality may instead be a nitrogen heterocyclic group, e.g. pyridine. Thus suitable R_2 groups may be



It is preferred that at least one of R_6 and R_7 be other than hydrogen and that R_6, if present, is preferably a substituent containing one or more polar hydrogens such as hydroxy, amino, alkylamino, aminoalkyl, alkylaminoalkyl, aminocarbonyl, alkylaminocarbonyl, alkylcarboxyamino, hydrazo and alkylhydrazo; alternatively R_6 and R_7 are joined together in the formation of a naphthyl or indolyl or azaindolyl or diazaindolyl group.

In a further preferred embodiment the compounds of the invention are of formula (A)



(A)

(wherein R_5, R_6, R_7, Ar, X-X, Lp_1, D_n are as hereinbefore defined; L_2 is a valence bond or an organic linker group containing 1 to 3 backbone atoms selected from C, N, O and S and R_8 and R_9 are hydrogen or taken together with the carbon atom to which they are attached form a carbonyl group). Again, in an alternative embodiment the phenyl derivative forming part of the R_2 functionality may instead be a nitrogen heterocyclic group, e.g. pyridine.

In one embodiment, L_2 comprises the backbone of an alpha amino acid, the lipophilic group being the side chain of the amino acid. The carboxyl part of the alpha amino acid may be optionally coupled via an amide bond to an amino acid or to a primary or secondary cyclic or acyclic alkyl amine or diamine or via an ester bond to primary or secondary alcohols.

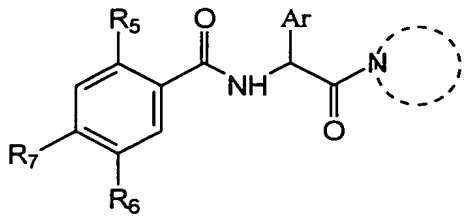
In one preferred embodiment R_8 and R_9 are hydrogen and L_2 is a OC=O or NHC=O group.

In a preferred embodiment, L_2 represents a valence

bond and the lipophilic group is bound directly to a carbonyl alpha to the alpha atom via a nitrogen atom which forms part of the lipophilic group. Suitable lipophilic groups in this case therefore include piperidinyl, pyrrolidinyl and piperazinyl. In a preferred embodiment the piperidine or piperazinyl group is further substituted by a phenyl, benzyl, phenoxy, piperidine, pyridine or benzoyl group, optionally substituted on the phenyl ring by one or more R_3 groups. In a more preferred embodiment a piperazine is substituted with a phenyl group substituted at the 2-position with an electron withdrawing group such as fluoro, nitro, triazolyl, cyano, alkoxycarbonyl, aminocarbonyl, aminosulphonyl, alkylaminosulphonyl and, especially preferred, alkylsulphonyl; and, at the 4-position, with hydrogen, fluoro, alkoxy or hydroxy. In another more preferred embodiment a piperidine is substituted at the 4-position with 4-piperidine which itself may be substituted on nitrogen by alkyl or aminocarbonylalkyl or alkylaminocarbonyl alkyl.

In a further embodiment, the lipophilic group has attached a group of the formula $-COOR_1$ or -CON-aminoacid or ester derivative thereof.

Particularly preferred compounds are those of formula (G)

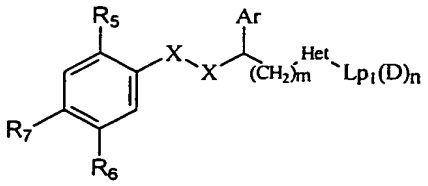


(G)

(wherein Ar, R_6 and R_7 are as hereinbefore defined, R_5 represents hydrogen or amino and ----- represents a cyclic group). Again, in an alternative embodiment the phenyl derivative forming part of the R_2 functionality may instead be a nitrogen heterocyclic group, e.g.

pyridine.

In another embodiment the group binding the alpha carbon atom to the lipophilic group comprises a heterocyclic group. Accordingly, preferred compounds of the invention also include those of formula (III)



(III)

(wherein R_5, R_6, R_7, Ar, X-X, Lp_1, D_n are as hereinbefore defined;

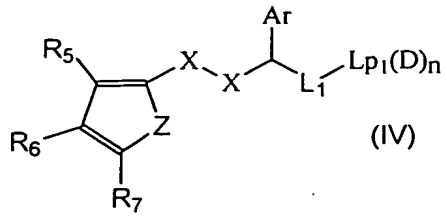
m is 0, 1 or 2;

Het is a 5 or 6-membered heterocyclic group interrupted by 1, 2 or 3 heteroatoms selected from O, N and S optionally substituted by a group R_3). Again, in an alternative embodiment the phenyl derivative forming part of the R_2 functionality may instead be a nitrogen heterocyclic group, e.g. pyridine.

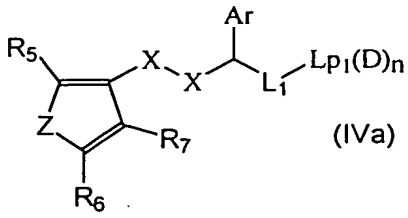
Where Het is a five membered ring, the two ring atoms at which it is connected are preferably separated by one ring atom. Where Het is a six-membered ring, the two ring atoms at which it is connected are preferably separated by one or two ring atoms. Representative heterocyclic groups include thiazole, oxazole, oxadiazole, triazole, thiadiazole or imidazole. Where the heterocyclic group is substituted by R_3 this is preferably a COOH or $COOR_1$ connected to the heterocycle via a valence bond or alkylene chain.

In a further embodiment, the lipophilic group has attached a group of the formula $-COOR_1$ or -CON-aminoacid or ester derivative thereof.

In an alternative embodiment the main aromatic R_2 ring in the compounds of the invention is a five membered aromatic ring of formula (IV) or (IVa)

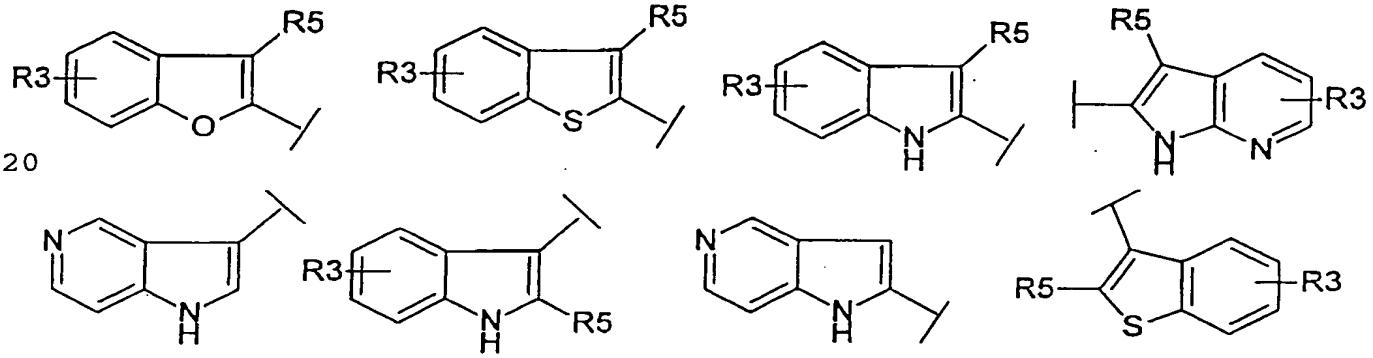


(IV)

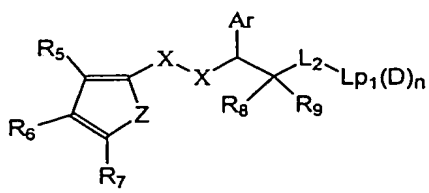


(IVa)

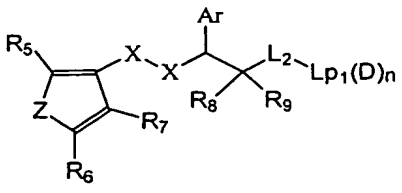
(wherein R_5, R_6, R_7, X-X, Ar, L_1, Lp_1, D and n are as hereinbefore described for formula (II) and Z represents N, O or S). It is preferred that at least one of R_6 and R_7 be other than hydrogen, or that R_6 and R_7 taken together enable the formation of an indolyl, or azaindolyl group or diazaindolyl group. Preferences for other substituents are as for formula (A) above. Examples of possible fused systems are given below.



Hence in a preferred embodiment the compounds of the invention are of formula C or D



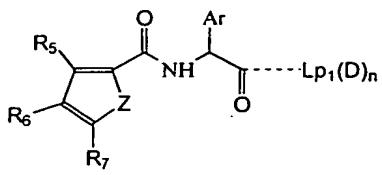
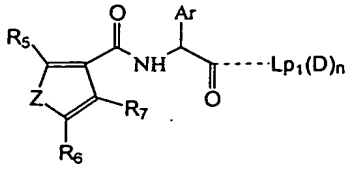
(C)



(D)

(wherein R_5, R_6, R_7, Ar, X-X, Z, R_8, R_9, L_2 Lp_1, D_n are as hereinbefore defined) preferences for Ar, X-X, R_8, R_9, L_2 Lp_1, D_n are as for formula (A) above; or compounds of

formula E or F:

(E)  (F) 

(wherein Lp_1 is connected to the carbonyl via a nitrogen atom, R_6, R_7, Ar, Z, Lp_1, D_n are as hereinbefore defined and R_5 is hydrogen or amino) preferences for Ar, Lp_1, D_n are as for formula (A) above.

The compounds of the invention may be prepared by conventional chemical synthetic routes, e.g. by amide bond formation to couple the aromatic function to the alpha atom and to couple the lipophilic function to the alpha atom. Where the alpha atom is a carbon, the cyclic group-alpha atom combination may conveniently derive from an alpha amino acid with the aromatic deriving from for example an acid derivative of a compound based on R_2, e.g. o-amino-benzoic acid. Amide formation from such reagents (in which any amino or hydroxyl function may if desired be protected during some or all of the synthesis steps) yields a compound of formula (V).

$$R_2\text{-CONH-CH(Cy)-COOH} \qquad (V)$$

(where Cy and R_2 are as defined above).

The lipophilic group (and optionally simultaneously the hydrogen bond donor) may then conveniently be introduced by reaction of a compound of formula (V) (or another analogous carboxylic acid) optionally after transformation into an activated form, e.g. an acid chloride or active ester, with a lipophilic group carrying an amine, hydroxylamine, hydrazine or hydroxyl group, e.g. to produce compounds with linkages of -CO-

NR_1-, -CO-NR_1-O-, -CO-NR_1-NR_1- and -CO-O- from the alpha atom (where it is a carbon) to the lipophilic group. Where Y and L taken together form a cyclic amide group the lipophilic group can be conveniently introduced by reacting the compound of formula (V) with a lipophilic group carrying a secondary amine with an active side chain. Cyclisation can be base induced via nucleophilic attack of the alpha atom on a leaving group on the active side chain. If necessary the amide linkage can be reduced using an appropriate reducing agent employing the necessary protection depending on whether concurrent reduction of the carboxylic acid moiety is also desired. Alternatively a compound of formula V or another analogous carboxylic acid may be transformed into an alcohol by reaction with isobutylchloroformate and reduction with sodium borohydride.

Such an alcohol, e.g. of formula VI

$$R_2 - CONH - CH(Cy)CH_2OH \qquad (VI),$$

can be reacted to introduce the lipophilic group by reactions such as:

alkylation with an alkyl halide in the presence of a base;

reaction with diethyl azodicarboxylate/triphenylphosphine and a hydroxylated aryl compound;

by reaction with an activated carboxylic acid (e.g. an acid chloride) or with a carboxylic acid and diethylazodicarboxylate/triphenylphosphine;

by reaction with an isocyanate; and

by treatment with methanesulphonyl chloride or trifluoromethanesulphonic anhydride and reaction with an amine, or with a thiol optionally followed by oxidation, e.g. with potassium metaperiodate or hydrogen peroxide.

In this way compounds with linkages of -CH_2-O-, -CH_2-O-CO-, -CH_2-O-CO-NR_1-, -CH_2-NR_1-, -CH_2-S-, -CH_2-SO-

and $-CH_2-SO_2-$ between the alpha carbon and the lipophilic group may be produced.

Alternatively the alcohol can be oxidized to form a corresponding aldehyde (e.g. by oxidation with manganese dioxide or DMSO/oxalyl chloride or DMSO/SO_3 or Dess-Martin reagent) which may be reacted to introduce the lipophilic group by reactions such as:

reaction with Wittig reagents or Horner-Emmons reagents, optionally followed by reduction of the resulting carbon:carbon double bond using H_2/Pd-carbon;

reaction with an organometallic, eg a Grignard reagent, optionally followed by reaction on the resulting hydroxyl group, such as oxidation (eg with MnO_2, DMSO/oxalyl chloride or Dess-Mártin reagent), alkylation (eg with an alkyl halide in the presence of a base in a solvent such as DMF), arylation (eg with diethylazo dicarboxylate/triphenyl phosphine and a hydroxyaryl compound), ester formation (eg with an acid chloride or with a carboxylic acid and diethylazido dicarboxylate/triphenyl phosphine), or carbamate formation (eg with an isocyanate);

by reaction with an amine followed by reduction, e.g. with sodium cyanoborohydride;

by reaction with a hydrazine; or

by reaction with a carbazide.

In this way compounds with linkages of $-CH=CR_1-$, $-CH_2-CHR_1-$, $-CHOH-$, $-CHR_1-O-$, $-CHR_1-O-CO-$, $-CHR_1-O-CO-NR_1-$, $-CO-$, $-CH_2-NR_1-$, $-CH=N-NR_1-$ and $-CH=N-NR_1-CO-NR_1-$ between the alpha carbon and the lipophilic group may be produced.

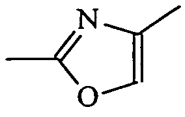
The transformation of alcohol to amine referred to above may be used to produce an amine reagent for lipophilic group introduction, e.g. a compound $R_2-CONH-CH(Cy)-CH_2-NR_1H$.

Such an amine reagent may be reacted to introduce the lipophilic group, e.g. by acylation with an acid halide or activated ester, by reaction with isocyanate,

by reaction with an isothiocyanate, or by reaction with a sulphonyl chloride. In this way compounds with linkages of $-CH_2NR_1-CO-$, $-CH_2-NR_1-CO-NR_1-$, $-CH_2NR_1-CS-NR_1-$ and $-CH_2NR_1-SO_2-$ between the alpha carbon and the lipophilic groups may be produced.

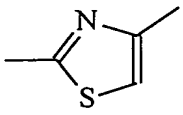
The transformation of acid to amide referred to above may be used to produce an amide reagent for introduction of the lipophilic group, e.g. a compound $R_2-CONH-CH(Cy)-CON(R_1)_2$.

Such amides may be reacted to introduce lipophilic groups, e.g. by reaction with a haloketone (e.g. phenacyl bromide). This provides a linkage

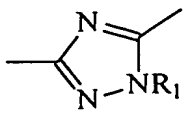


from alpha carbon to lipophilic group.

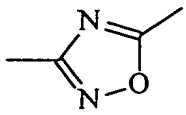
Analogously the amide may be transformed to a thioamide by reaction with Lawesson's reagent and then reacted with a haloketone to form a linkage



The amide reagent may likewise be transformed to a nitrile reagent by dehydration, e.g. with trifluoroacetic anhydride. The nitrile reagent may be reacted with hydrazine then with acyl halide and then cyclized, (e.g. with trifluoroacetic anhydride) to produce a linkage

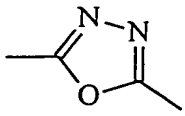


Alternatively it may be treated with hydroxylamine then reacted with acyl halide and cyclized (e.g. with trifluoroacetic anhydride) to produce a linkage



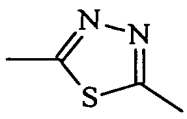
The hydrazide produced by reaction of a carboxylic acid reagent with hydrazine discussed above may likewise be used as a reagent for lipophilic group introduction, e.g. as a compound of formula R_2-CONH-CH(Cy)-CO-NR_1-N$(R_1)_2$.

Thus the hydrazide reagent can be reacted with an acyl halide and cyclized, e.g. with trifluoroacetic anhydride to yield a linkage

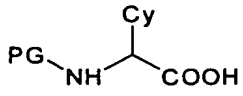


or reacted with an acyl halide or an isocyanate to yield linkages -CO-NR_1-NR_1-CO- and -CO-NR_1-NR_1-CO-NR_1- respectively.

Alternatively the hydrazide may be transformed by reaction with Lawesson's reagent and then reacted with an acyl halide and cyclized (e.g. with trifluoroacetic anhydride) to produce the linkage



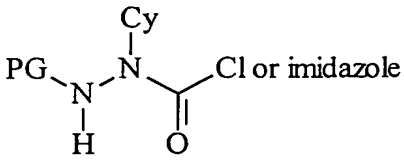
An alternative route to these compounds is to carry out any of the above chemical reactions to incorporate the lipophilic group (an optional H bond donor) into a protected intermediate such as a compound of formula (VII).



PG=Protecting group

The protecting group may then be removed before coupling of the for example o-amino benzoic acid (optionally protected).

A starting reagent for lipophilic group introduction where the alpha atom is nitrogen may be produced for example by reaction of a beta protected hydrazine (such protection to be chosen as to be compatible with the subsequent reagents to be employed) with phosgene, diphosgene, triphosgene or N,N'carbonyl diimidazole to give a reactive compound of the type:



PG = Protecting group

This intermediate may be used as has been described above for the carboxylic starting reagents where the alpha atom is carbon.

Removal of the protecting group by standard methods and coupling with an activated aryl carboxylic acid will give compounds of the type

$$R_2\text{-}CONH\text{-}N(Cy)\text{-}L\text{-}Lp(D)_n$$

(where R_2, X, Y, Cy, L, Lp and D are as defined above).

Thus viewed from a further aspect the invention provides a process for the preparation of a compound according to the invention which process comprises coupling a lipophilic group to a compound of formula (VIII)

$$R_2\text{-}(X)_2\text{-}Y(Cy)\text{-}Z_1 \qquad \text{(VIII)}$$

(wherein R_2, X, Y and Cy are as defined above and Z_1 is a reactive functional group), and optionally subsequently coupling a hydrogen bond donor group to said lipophilic group.

The compounds of the invention may be administered by any convenient route, e.g. into the gastrointestinal tract (e.g. rectally or orally), the nose, lungs, musculature or vasculature or transdermally. The compounds may be administered in any convenient administrative form, e.g. tablets, powders, capsules, solutions, dispersions, suspensions, syrups, sprays, suppositories, gels, emulsions, patches etc. Such compositions may contain components conventional in pharmaceutical preparations, e.g. diluents, carriers, pH modifiers, sweeteners, bulking agents, and further active agents. Preferably the compositions will be sterile and in a solution or suspension form suitable for injection or infusion. Such compositions form a further aspect of the invention.

Viewed from this aspect the invention provides a pharmaceutical composition comprising a serine protease inhibitor according to the invention together with at least one pharmaceutically acceptable carrier or excipient.

Viewed from a further aspect the invention provides the use of a serine protease inhibitor according to the invention for the manufacture of a medicament for use in a method of treatment of the human or non-human animal body (e.g. a mammalian, avian or reptilian body) to combat (i.e. treat or prevent) a condition responsive to said inhibitor.

Viewed from a further aspect the invention provides a method of treatment of the human or non-human animal body (e.g. a mammalian, avian or reptilian body) to combat a condition responsive to a serine protease inhibitor (e.g. a condition such as a thrombotic disorder responsive to a factor Xa inhibitor), said method comprising administering to said body an effective amount of a serine protease inhibitor according to the invention.

The dosage of the inhibitor compound of the

invention will depend upon the nature and severity of the condition being treated, the administration route and the size and species of the patient. However in general, quantities of from 0.01 to 100 μmol/kg bodyweight will be administered.

All publications referred to herein are hereby incorporated by reference.

The invention will now be described further with reference to the following non-limiting Examples.

Experimental

Abbreviations used follow IUPAC-IUB nomenclature. Additional abbreviations are Hplc, high-performance liquid chromatography; DMF, dimethylformamide; DCM, dichloromethane; HAOT, 1-hydroxy-7-azabenzotriazole; HATU, [O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate]; Fmoc, 9-Fluorenylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazole; TBTU, 2-(1H-(benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate; EDCI, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DIPEA, diisopropylethylamine; Boc, tertiary butyloxycarbonyl; DIPCI, diisopropylcarbodiimide; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; TEA, triethylamine; Rink linker, p-[(R,S)-α-[1-(9H-Fluoren-9-yl)methoxyformamido]-2,4-dimethoxybenzyl]phenyl acetic acid; TFA, trifluoroacetic acid; MALDI-TOF, Matrix assisted laser desorption ionisation - time of flight mass spectrometry, RT, retention time. Unless otherwise indicated amino acid derivatives, resins and coupling reagents were obtained from Novabiochem (Nottingham, UK) and other solvents and reagents from Rathburn (Walkerburn, UK) or Aldrich (Gillingham, UK) and were used without further purification. All solution concentrations are expressed as %Vol./%Vol. unless otherwise stated.

Purification: Purification was by gradient reverse phase Hplc on a Waters Deltaprep 4000 at a flow rate of 50 ml/min. using a Deltapak C18 radial compression column (40 mm x 210 mm, 10-15 mm particle size). Eluant A consisted of aqTFA (0.1%) and eluant B 90% MeCN in aqTFA(0.1%) with gradient elution (Gradient 1, 0 min. 20%B then 20% to 100% over 36 min., Gradient 2, 0 min. 5%B for 1 min. then 5%B to 20%B over 4 min., then 20% to 60% over 32 min. or Gradient 3, 0 min. 20%B then 20%

to 100% over 15 min.). Fractions were analysed by analytical Hplc and MALDI-TOF before pooling those with >95% purity for lyophilisation.

Analysis: Analytical Hplc was on a Shimadzu LC6 gradient system equipped with an autosampler, a variable wavelength detector at flow rates of 0.4 ml/ min. Eluents A and B as for preparative Hplc . Columns used were Techogel15 C18 (2x150mm)(Hplc Technology), Magellan C8 column (2.1x150 mm, 5μm particle size) (Phenomenex))Purified products were further analysed by MALDI-TOF and nmr.

Synthesis of inhibitors

Method 1: Using a solid phase strategy on a Protein Technologies, Symphony Multiple Peptide Synthesiser by attachment of bis amino compounds to Peg-trityl chloride resin: Trityl chloride resin was typically treated with greater than 2 fold excess of the di-amine in dry DCM .The resin was further modified by the attachment of acids. Activation of Fmoc protected amino acid (2-5eq) was by TBTU/ DIPEA, all couplings (minimum 120 min.) were carried out in DMF. Deprotection of the Fmoc group was achieved with 20% piperidine in DMF. In the next stage other acid substituents were added as the HOBt or HOAt esters either by activation with HBTU/HATU or HATU/EDCI with or without Boc protection of amino groups. Cleavage of the products from the resin was by treatment (30 min., ambient) with 10% triethylsilane in TFA, filtration, evaporation and trituration with diethylether.

Synthesis using the Symphony Multiple Peptide Synthesiser.

The Symphony Multiple Peptide Synthesiser is charged with DMF, DCM, TBTU in DMF(450 mM), DIPEA in DMF (900 mM), 20% piperidine in DMF. Resins are held in plastic reaction vessels that allow the introduction of reagents and solvents and nitrogen for agitation or air drying.

A typical synthesis cycle on the Symphony is as follows:-

The reaction vessel containing the resin (0.1 mmol) is charged with the Fmoc protected amino acid (0.5 mmol) and then this is dissolved in DMF (2.5ml), treated with TBTU (0.56 mmol, 1.25ml) and DIPEA (1.1 mmol, 1.25ml) and agitated with nitrogen for 2 hours (agitation times may vary). After coupling the resin is washed with DMF (6x 5ml) then deprotected with 20% piperidine in DMF (2x 5ml for 1 min.each, then 1x 5ml for 8 min.) the resin is then washed with DMF (6x 5ml).

Example 1.
2-Amino-4-chlorobenzoyl-D-phenylglycine 4,4'bispiperidinamide

4,4-Bipiperidine.dihydrochloride (4mmol,1g) was dissolved in water (5ml) and 2M sodium hydroxide solution (10mmol, 5ml) added. The solution was extracted with ethylacetate (2x 50ml) the combined extracts were washed with water, dried over anhydrous sodium carbonate, filtered and evaporated to give the 4,4 bipiperidine (0.35g) as a white solid. The 4,4 bipiperidine was dissolved in dry DMF (2ml) and added to Peg-tritylchloride resin (0.95 mmol/g, 1.5g) pre swollen in dry DCM (10ml). After 2h the resin was washed with DCM (6x5ml), DMF (6x5ml) and DCM (6x5ml). The resin was then air dried to allow aliquots to be taken.

The 4,4 bipiperidine trityl resin (0.1 mmol) was treated

with Fmoc-D-Phenylglycine (0.5 mmol, 187mg), DMF(2.5ml), TBTU in DMF(1.25ml of a 450mM solution) and DIPEA in DMF (1.25ml of a 900 mM solution). The mixture was agitated with nitrogen for 2 hours. Deprotection and washing as above.

A solution of 4-chloroanthranilic acid (87mg 0.5mmole) in dry dimethylformamide (DMF) was treated successively with HOAt (102mg 0.75mmole) and EDCI (115mg 0.6mmole) and stirred at room temperature for 10min. The mixture was transferred to the reaction vessel on the Symphony and agitated for 2 hours with nitrogen. The resin was washed with DMF (6x5ml), DCM (6x5ml) and air dried. The product was cleaved from the resin with 10% triethylsilane in TFA (10ml) for 30 minutes, the resin filtered off and the TFA solution evaporated to dryness and triturated with diethyl ether to give the crude product. The crude product was dissolved in water (10ml), filtered and purified by preparative reverse phase Hplc.

^{1}H nmr (CD_3CN) 7.30 (6H,m); 6.60 (1H,s); 6.55 (1H,d); 5.85 (1H, s); 4.40 (1H,m); 3.75 (1H, m); 2.30-2.95 (6H, m); 1.60 (4H, m); 1.10 (6H, m) MS TOF 456 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.77 min.

Example 2.
2-Amino-5-bromobenzoyl-D-phenylglycine 4,4'bispiperidinamide
^{1}H nmr (CD_3CN) 7.30 (7H,m); 6.50 (1H,d); 5.85 (1H, s); 4.40 (1H,m); 3.75 (1H, m); 2.30-2.95 (6H, m); 1.60 (4H, m); 1.10 (6H, m) MS TOF 500 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.31 min.

Example 3.

2-Amino-4-methylbenzoyl-D-phenylglycine 4,4'bispiperidinamide

^{1}H nmr (CD_3CN) 7.30 (6H,m); 6.50 (1H,s); 6.45 (1H,d); 5.80 (1H, s); 4.40 (1H,m); 3.75 (1H, m); 2.30-2.95 (6H, m); 2.05 (3H,s); 1.60 (4H, m); 1.10 (6H, m) MS TOF 436 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 9.22 min.

Example 4.

2-Amino-5-methylbenzoyl-D-phenylglycine 4,4'bispiperidinamide

^{1}H nmr (CD_3CN) 7.30 (7H,m); 6.50 (1H,d); 5.85 (1H, s); 4.40 (1H,m); 3.75 (1H, m); 2.30-2.95 (6H, m); 1.60 (4H, m); 1.10 (6H, m). MS TOF 436 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 8.74 min.

Example 5.

2-Amino-5-methoxybenzoyl-D-phenylglycine 4,4'bispiperidinamide

^{1}H nmr (CD_3CN) 7.55 (6H,m); 7.30 (1H,d); 6.95 (1H,m); 6.15 (1H, s); 4.40 (1H,m); 3.75 (1H, m); 3.60 (3H, s); 2.30-2.95 (6H, m); 2.20 (3H, s); 1.60 (4H, m); 1.10 (6H, m) MS TOF 452 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 8.20 min.

Example 6.

2-Dimethylaminobenzoyl-D-phenylglycine 4,4'bispiperidinamide

^{1}H nmr (CD_3CN) 7.80 (1H,d); 7.65 (2H,m); 7.30 (6H,m); 5.85 (1H, s); 4.40 (1H,m); 3.75 (1H, m); 3.10 (6H, s); 2.30-2.95 (6H, m); 1.60 (4H, m); 1.10 (6H, m) MS TOF 450 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 9.57 min.

Example 7.

3-Methylbenzoyl-D-phenylglycine 4,4'bispiperidinamide

^{1}H nmr (CD_3CN) 7.40 (2H,m); 7.30 (7H,m); 5.85 (1H, s); 4.40 (1H,m); 3.75 (1H, m); 2.30-2.95 (6H, m); 2.20 (3H, s); 1.60 (4H, m); 1.10 (6H, m) MS TOF 421 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.68 min.

Example 8.

4-Methylbenzoyl-D-phenylglycine 4,4'bispiperidinamide

^{1}H nmr (CD_3CN) 7.55 (2H,m); 7.30 (5H,m); 7.10 (2H,m); 5.85 (1H, s); 4.40 (1H,m); 3.75 (1H, m); 2.30-2.95 (6H, m); 2.20 (3H,s); 1.60 (4H, m); 1.10 (6H, m) MS TOF 420 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.61 min.

Example 9.

3-Amino-2-naphthoyl-D-phenylglycine 4,4'bispiperidinamide

^{1}H nmr (CD_3CN) 7.90 (1H,d); 7.60 (1H,d); 7.40 (1H,m); 7.30 (6H,m); 7.05 (1H,m); 6.90 (1H,s); 5.85 (1H, s); 4.40 (1H,m); 3.75 (1H, m); 2.30-2.95 (6H, m); 1.60 (4H, m); 1.10 (6H, m) MS TOF 471 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 9.87 min.

Example 10.

3-Aminobenzoyl-D-phenylglycine 4,4'bispiperidinamide

MS TOF 421 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 9.06 min.

Example 11.

2-Aminobenzoyl-D-phenylglycine 4,4'bispiperidinamide

MS TOF 421 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 9.00 min.

Example 12.

2-Amino-4-fluorobenzoyl-D-phenylglycine 4,4'bispiperidinamide

MS TOF 440 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 9.23 min.

Example 13.

2-Amino-5-fluorobenzoyl-D-phenylglycine 4,4'bispiperidinamide

MS TOF 440 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 9.14 min.

Example 14.

2-Amino-4-nitrobenzoyl-D-phenylglycine 4,4'bispiperidinamide

MS TOF 467 ($M+1^+$). Hplc (Magellan C8, Gradient 3,

water/acetonitrile/TFA) rt 10.59 min.

Example 15.

2-Amino-5-nitrobenzoyl-D-phenylglycine 4,4'bispiperidinamide

MS TOF ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.57 min.

Example 16.

2-Amino-4,5-dimethoxybenzoyl-D-phenylglycine 4,4'bispiperidinamide

MS TOF 481 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.67 min.

Example 17.

Benzoyl-D-phenylglycine 4,4'bispiperidinamide

MS TOF 407 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 9.88 min.

Example 18.

4-Chlorobenzoyl-D-phenylglycine 4,4'bispiperidinamide

MS TOF 441 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.89 min.

Example 19.

2-Hydroxybenzoyl-D-phenylglycine 4,4'bispiperidinamide

MS TOF 423 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 8.97 min.

Method 2: By solution phase strategy: Typically an activated Boc-amino acid was treated with an amine (primary or secondary) or alcohol (1eq.). Activation of Boc protected amino acid was by HATU or TBTU/ DIPEA(1:2), all couplings (minimum 120 min.) were carried out in DMF. After an aqueous work up the deprotection of the Boc group was achieved with TFA. Other acid substituents were added as the HOBt or HOAt esters either by activation with HBTU/HATU, EDC or DIPCI with or without Boc protection of amino groups. The final products were purified by preparative reverse phase Hplc.

Example 20.
3-Hydroxymethylbenzoyl-D-phenylglycine-4-methylbenzylamide

Boc D-phenylglycine (251 mg, 1 mmol.) was dissolved in DMF(3ml) with HATU (380 mg., 1 mmol.) and DIPEA(350μl ., 2 mmol.). To this mixture was added 4-methylbenzylamine(121mg., 1 mmol.) and DIPEA (170μl., 1 mmol.). The mixture was stirred overnight. The mixture was then taken up into ethylacetate and washed with water, sodium carbonate solution, water, 10% hydrochloric acid solution and water. The ethylacetate was evaporated without drying and treated immediately with TFA for 30 min. The TFA was then evaporated to dryness and the product triturated with diethylether. TEA(1ml) was added and evaporated to dryness. A solution of 3-hydroxymethylbenzoic acid (76mg , 0.5mmole) in dry dimethylformamide (DMF) was treated with TBTU (161mg., 0.5mmol.) and DIPEA (1.5 mmol.). The mixture was then added to the D-phenylglycine-4-methylbenzylamide (0.5mmol.) and stirred overnight. The crude product was dissolved in water/acetonitrile (20ml), filtered and purified by preparative Hplc to yield pure product.

^{1}H nmr (CD_3CN) 7.75 (1H, m); 7.65 (2H, m); 7.30 (7H, broad m); 6.80 (3H, m); 5.40 (1H, s); 4.45 (2H,s); 4.10 (2H, m); 2.10 (3H, s). MS TOF 389 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 13.51 min.

Compounds made by the above method:-

Example 21.
3-Hydroxybenzoyl-D-phenylglycine-4-methylbenzylamide

^{1}H nmr (CD_3CN) 7.75 (1H, m); 7.40 (2H, m); 7.30 (5H, broad m); 6.95 (5H, m); 5.40 (1H, s); 4.20 (2H, m); 2.20 (3H, s). MS TOF 375 ($M+1^+$). Hplc (Magellan C8, Gradient

3, water/acetonitrile/TFA) rt 12.28 min.

Example 22.

3-Aminobenzoyl-D-phenylglycine-4-methylbenzylamide

^{1}H nmr (CD_3CN) 7.70-7.30 (13H, broad m); 5.65 (1H, s); 4.35 (2H, m); 2.25 (3H, s). MS TOF 374 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 10.44 min.

Example 23.

3-Amidobenzoyl-D-phenylglycine-4-methylbenzylamide

^{1}H nmr (CD_3CN) 8.40 (1H, m); 8.20 (2H, m); 7.60 (6H, broad m); 7.20 (4H, m); 5.75 (1H, s); 4.50 (2H, m); 2.40 (3H, s). MS TOF 402 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.16 min.

Example 24.

3-Aminomethylbenzoyl-D-phenylglycine-4-methylbenzylamide

^{1}H nmr (CD_3CN) 7.80 (2H, m); 7.45 (5H, m); 7.30 (2H, m); 6.95 (4H, m); 5.55 (1H, s); 4.25 (2H,s); 4.05 (2H, s); 2.20 (3H, s). MS TOF 388 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 12.28 min.

Example 25.

3-Amidobenzoyl-D-phenylglycine-4-(aminomethyl)benzylamide

^{1}H nmr (CD_3CN) 8.20 (1H, s); 7.95 (2H, m); 7.60 (1H,m); 7.30 (5H, broad m); 6.95 (5H, m); 5.40 (1H, s); 4.20 (2H, m); 2.20 (3H, s). MS TOF 417 ($M+1^+$). Hplc (Magellan C8, Gradient 2, water/acetonitrile/TFA) rt 14.05 min.

Example 26.

3-Aminomethylbenzoyl-D-phenylglycine-4-aminomethylcyclohexyl methylamide

^{1}H nmr (CD_3CN) 7.95 (2H, m); 7.80 (2H, m); 7.50 (5H, m); 5.65 (1H, s); 4.45 (2H, s); 3.30 (2H, m); 3.00 (2H,m); 2.00-1.00 (10H,m). MS TOF 409 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 12.68 min.

Example 27.

2-Amino-N-[1-(ethoxycarbonyl)-1-(phenyl)methyl]benzimidazole-5-carboxamide

^{1}H nmr (CD_3CN) 7.80 (1H, s); 7.55 (1H, d); 7.40 (5H, m);

7.20 (1H,d); 5.85 (1H, s); 4.15 (2H, m); 1.25 (3H, m). MS TOF 339 ($M+1^+$). Hplc (Magellan C8, Gradient 2, water/acetonitrile/TFA) rt 17.05 min.

Example 28.

3-Aminomethylbenzoyl-D-phenylglycine-1-adamantylamide

^1H nmr (CD_3CN) 7.95 (1H, s); 7.85 (2H, d); 7.60 (1H, m); 7.50 (2H,m); 7.40 (3H,m); 5.65 (1H, s); 4.20 (2H, s); 2.50-1.50 (15H,m). MS TOF 418 ($M+1^+$). Hplc (Magellan C8, Gradient 1, water/acetonitrile/TFA) rt 18.36 min.

Example 29.

2-Aminobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (DMSO) 7.65 (3H, m); 7.45 (1H, m); 7.35 (5H, m);7.15 (1H,m); 6.65 (1H,d); 6.55 (1H,m); 6.05 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 511 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 13.43 min.

Example 30.

2-Amino-4-chlorobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (DMSO) 7.55 (3H, m); 7.45 (1H, m); 7.35 (5H, m);7.15 (1H,m); 6.75 (1H,s); 6.55 (1H,d); 6.05 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 546 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 15.18 min.

Example 31.

2-Amino-5-fluorobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 7.75 (1H, m); 7.60 (1H, m); 7.25 (6H, m);7.15 (1H,m); 6.90 (1H,m); 6.75 (1H,m); 5.85 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 529 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 13.87 min.

Example 32.

2-Amino-4-methylbenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (DMSO) 7.55 (3H, m); 7.45 (2H, m); 7.35 (5H, m);

6.65 (1H,s); 6.35 (1H,d); 6.05 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m) 2.15 (3H,s);. MS TOF 525 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 13.12 min.

Example 33.

2-Amino-5-methylbenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 7.75 (1H, m); 7.60 (1H, m); 7.25 (6H, m);7.15 (1H,m); 6.90 (1H,m); 6.75 (1H,m); 5.85 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m) 2.30 (3H,s). MS TOF 525 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 12.84 min.

Example 34.

2-Amino-4-nitrobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 7.75 (2H, m); 7.55 (1H, m); 7.35 (7H, m); 7.25 (1H,m); 5.80 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 556 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 15.35 min.

Example 35.

2-Amino-5-nitrobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 8.25 (1H, d); 7.85 (1H, m); 7.55 (1H, m); 7.25 (7H, m); 7.05 (1H,m); 5.80 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 556 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 15.08 min.

Example 36.

2-Amino-5-cyanobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 7.65 (4H, m); 7.25 (6H, m); 6.65 (1H,d); 5.80 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 536 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 14.89 min.

Example 37.

2,5-Diaminobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 7.70 (1H, d); 7.45 (7H, m); 6.85 (1H, s);

6.55 (1H, m); 6.55 (1H,m); 5.90 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 526 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.82 min.

Example 38.

2-Amino-4,5-dimethoxybenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 7.65 (2H, m); 7.35 (2H, m); 7.25 (5H, m); 6.75 (1H,d); 6.15 (1H, d);5.80 (1H, s); 3.60 (3H,s); 3.50 (3H,s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 571 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 12.84 min.

Example 39.

Benzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 7.75 (2H, m); 7.70 (1H, m); 7.40 (10H, m); 6.05 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 496 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 12.84 min.

Example 40.

2-Methylaminobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 7.75 (1H, m); 7.65 (1H, d); 7.50 (1H, d); 7.45 (2H, m); 7.30 (5H, m); 6.80 (1H, d); 6.70 (1H, m); 6.00 (1H, s); 3.15 (3H,s); 2.80 (3H,s); 3.00-2.00 (8H,m). MS TOF 525 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 14.63 min.

Example 41.

2-Dimethylaminobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 7.85 (1H, d); 7.50 (2H, m); 7.45 (3H, m); 7.30 (6H, m); 6.00 (1H, s); 3.15 (3H,s); 2.80 (6H,s); 3.00-2.00 (8H,m). MS TOF 539 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 12.58 min.

Example 42.

3-Aminobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 7.85 (1H, m); 7.60 (1H, m); 7.50 (2H, m);

7.30 (7H, m); 7.05 (1H, d); 6.05 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 511 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.32 min.

Example 43.

4-Aminobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 7.95 (1H, d); 7.80-7.45 (10H, broad m); 7.35 (1H,d); 6.20 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 511 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 12.05 min.

Example 44.

3,4 Diaminobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 7.75 (1H, d); 7.40-7.15 (9H, broad m); 6.55 (1H,d); 6.00 (1H, s); 3.15 (3H,s); 3.00-2.00 (8H,m). MS TOF 540 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.30 min.

Example 45.

3-Chlorobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 7.85 (1H, m); 7.80 (1H, s); 7.60 (2H, m); 7.30 (8H, m); 6.00 (1H, s); 3.20 (3H,s); 3.00-2.00 (8H,m). MS TOF 531 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 15.40 min.

Example 46.

4-Chlorobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 7.95 (1H, m); 7.75 (2H, m); 7.60 (1H, m); 7.40 (8H, m); 6.05 (1H, s); 3.25 (3H,s); 3.00-2.00 (8H,m). MS TOF 531 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 16.54 min.

Example 47.

3-Amino-4-chlorobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 8.05 (1H, m); 7.80 (1H, m); 7.70 (1H, s); 7.20-7.60 (8H, broad m); 6.05 (1H, s); 3.25 (3H,s); 3.00-2.00 (8H,m). MS TOF 546 ($M+1^+$). Hplc (Magellan C8,

Gradient 3, water/acetonitrile/TFA) rt 14.53 min.

Example 48.

4-Bromobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^{1}H nmr (CD_3CN) 7.85 (1H, m); 7.65 (2H, m); 7.60 (2H, d); 7.45 (2H, d); 7.30 (5H, m); 6.00 (1H, s); 3.20 (3H,s); 3.00-2.00 (8H,m). MS TOF 576 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 15.94 min.

Example 49.

4-Iodobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^{1}H nmr (CD_3CN)); 7.75 (2H, m); 7.65 (1H, m 7.55 (2H, d); 7.45 (2H, d); 7.30 (5H, m); 5.95 (1H, s); 3.20 (3H,s); 3.00-2.00 (8H,m). MS TOF 622 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 15.96 min.

Example 50.

3-Amino-4-methylbenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^{1}H nmr ($CDCl_3$) 7.95 (1H, s); 7.60 (1H, d); 7.45 (1H, d); 7.40-7.15 (8H, broad m); 6.00 (1H, s); 3.15 (3H,s); 3.00-2.50 (8H,m) 2.20 (3H, s). MS TOF 525 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.71 min.

Example 51.

4-Methoxybenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^{1}H nmr (CD_3CN) 7.85 (2H, d); 7.65 (1H, m); 7.50 (2H, m); 7.40 (5H, m); 6.80 (2H, d); 6.00 (1H, s); 3.80 (3H, s); 3.20 (3H,s); 3.00-2.00 (8H,m). MS TOF 526 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 14.63 min.

Example 52.

3-Amino-4-methoxybenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^{1}H nmr ($CDCl_3$) 7.90 (1H, m); 7.75 (1H, d); 7.60 (2H, m); 7.40-7.15 (6H, broad m); 7.45 (1H, d); 6.10 (1H, s); 3.95 (3H, s); 3.35 (3H,s); 3.00-2.50 (8H,m). MS TOF 541

($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.78 min.

Example 53.

3,4-Dihydroxybenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 7.55 (1H, m); 7.45 (1H, d); 7.25 (2H, m); 7.15 (5H, m); 7.00 (1H, d);6.60 (1H, d); 5.80 (1H, s); 3.05 (3H,s); 3.00-2.50 (8H,m). MS TOF 541 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.78 min.

Example 54.

Naphth-2-oyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 8.35 (1H, s); 8.00 (1H, d); 7.85 (5H, m); 7.45 (4H, m); 7.25 (4H, m); 6.10 (1H, s); 3.20 (3H,s); 3.00-2.50 (8H,m). MS TOF 546 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 16.66 min.

Example 55.

3-Aminonaphth-2-oyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 8.15 (1H, d); 8.00 (1H, s); 7.75 (2H, m); 7.65 (1H, d); 7.30 7.60 (9H, m); 6.10 (1H, s); 3.25 (3H,s); 3.00-2.50 (8H,m). MS TOF 561 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 13.90 min.

Example 56.

Thiophene-3-oyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 8.15 (1H, s); 7.95 (1H, m); 7.85 (1H, m); 7.60 (8H, m); 6.30 (1H, s); 3.45 (3H,s); 2.00-2.50 (8H,m). MS TOF 502 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 14.28 min.

Example 57.

Thiophene-2-oyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 7.65 (2H, m); 7.45 (1H, s); 7.30 (2H, m); 7.20 (5H, m); 6.95 (1H, m); 6.00 (1H, s); 3.05 (3H,s);

3.00-2.50 (8H,m). MS TOF 502 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 14.52 min.

Example 58.

5-Methyl thiophene-2-oyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr ($CDCl_3$) 7.70 (1H, m); 7.45 (2H, m); 7.35 (6H, m); 6.65 (1H, m); 6.00 (1H, s); 3.05 (3H,s); 3.00-2.50 (8H,m) 2.45 (3H, s). MS TOF 516 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 14.98 min.

Example 59.

Isoquinolin-7-oyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 9.50 (1H, s); 8.75 (1H, s); 8.55 (1H, d); 8.30 (1H, d); 8.10 (2H, m); 7.65 (1H, m); 7.45 (2H, m); 7.35 (5H, m); 6.10 (1H, s); 3.20 (3H,s); 3.00-2.50 (8H,m). MS TOF 547 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.39 min.

Example 60.

Pyridin-3-oyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 9.00 (1H, s); 8.70 (1H, d); 8.35 (1H, d); 8.10 (1H, m); 7.65 (2H, m); 7.45 (1H, m); 7.30 (5H, m); 6.00 (1H, s); 3.20 (3H,s); 3.00-2.50 (8H,m). MS TOF 497 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.99 min.

Example 61.

Indol-6-oyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

^1H nmr (CD_3CN) 7.95 (2H, m); 7.60 (2H, m); 7.50 (3H, m); 7.35 (5H, m);6.45 (1H, s); 6.05 (1H, s); 3.25 (3H,s); 3.00-2.50 (8H,m). MS TOF 535 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 15.44 min.

Example 62.

2,4-Diaminobenzoyl-D-phenylglycine-N-(4-fluoro-2-methylsulphonylphenyl)piperazinamide

MS TOF 526 ($M+1^+$). Hplc (Magellan C8, Gradient 3, water/acetonitrile/TFA) rt 11.89 min.

Assay protocols

Enzyme Inhibition assays:

Enzyme assays were carried out at room temperature in 0.1M phosphate buffer, pH7.4 according to the method of Tapparelli et al (J. Biol. Chem. 1993,268,4734-4741). Purified human factor Xa, trypsin, thrombin and plasmin were purchased from Alexis Corporation, Nottingham, UK. Urokinase was purchased from Calbiochem, Nottingham, UK. Chromogenic substrates for these enzymes; pefachrome-FXA, pefachrome-TRY, pefachrome-TH, pefachrome-PL and pefachrome-UK were purchased from Pentapharm AG, Basel, Switzerland. Product (*p*-nitroaniline) was quantified by adsorption at 405nm in 96 well microplates using a Dynatech MR5000 reader (Dynex Ltd, Billingshurst, UK). Km and Ki were calculated using SAS PROC NLIN (SAS Institute, Cary, NC, USA, Release 6.11) K_m values were determined as 100.9μM for factor Xa/pefachrome-FXA and 81.6μM for trypsin/pefachrome-TRY. Inhibitor stock solutions were prepared at 40mM in Me2SO and tested at 500μM, 50μM and 5μM. Accuracy of Ki measurements was confirmed by comparison with Ki values of known inhibitors of factor Xa and trypsin.

In agreement with published data, benzamidine inhibited factor Xa, trypsin, thrombin, plasmin and urokinase with Ki values of 155μM, 21μM, 330nM, 200nM and 100nM respectively. NAPAP inhibited thrombin with a Ki value of 3nM. Compounds of the invention were found to have activity in these assays.

Partial Thromboplastin Time Test Protocol

Venous blood was collected into 3.2% (0.109m) trisodium citrate vacutainer tubes at 1 volume of anticoagulant to

nine volumes of blood. The blood cells were separated by centrifugation at 700g for ten minutes to yield plasma, which was frozen at 70°C until required.
To perform the test, 100µl of plasma was pipetted into in a glass test tube, 1µl of test compound in DMSO was added, and allowed to warm to 37° over two minutes. 100µl of warm (37°) Manchester (tissue thromboplasin) reagent (Helena Biosciences, UK) was added, allowed to equilibrate for sixty seconds. 100µl of warm (37°) 25mM calcium chloride solution was added to initiate clotting. The test tube was tilted three times through a 90° angle every five seconds to mix the reagents and the time to clot formation recorded. Data from a series of observations and test compound concentrations are analysed by a SAS statistical analysis program and a CT2 (Concentration required to double clotting time) for each compound is generated.

Compounds of the invention were found to significantly elongate the partial thromboplastin time.

Example No.	Conc. necessary to double the prothrombin time (µM)
9	26
37	6.7
42	7.8
44	11
47	8.8
50	9.0
51	12
52	12

Compounds of the invention were found to be potent inhibitors of factor Xa.